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Review

Magnetic resonance imaging of postmortem human brain specimens: methodological considerations and prospects in psychoradiology

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Abstract

Ex vivo magnetic resonance imaging (MRI) has revolutionized psychoradiological research by enabling detailed structural and pathological assessments of the brain in conditions ranging from psychiatric disorders to neurodegenerative diseases. By providing high-resolution images of postmortem brain tissue, *ex vivo* MRI overcomes several limitations inherent in *in vivo* imaging, offering unparalleled insights into the underlying pathophysiology of mental disorders. This review critically summarizes the state-of-the-art *ex vivo* MRI methodologies for neuroanatomical mapping and pathological characterization in psychoradiology, while also establishing standardized specimen processing protocols. Furthermore, we explore the prospects of application in *ex vivo* MRI in schizophrenia, major depressive disorder and bipolar disorder, highlighting its role in understanding neuroanatomical alterations, disease progression, and the validation of *in vivo* neuroimaging biomarkers.

Keywords: psychoradiology; magnetic resonance imaging; *ex vivo* brain; psychiatric disorders

Introduction

Psychoradiology, a rapidly evolving field at the intersection of psychiatry, neurology, and neuroimaging, seeks to understand the brain structures and networks underlying psychiatric and neurodegenerative disorders. While *in vivo* magnetic resonance imaging (MRI) has been widely used in clinical practice to assess brain abnormalities associated with these conditions, *ex vivo* MRI has emerged as a complementary technique for providing more detailed microstructural insights. *Ex vivo* imaging involves scanning brain tissue that has been removed from the body, typically postmortem. *Ex vivo* human brain MRI yields extremely high-resolution anatomical images, surpassing the clinical resolution of 1–2 mm typically achieved in *in vivo* MRI conducted using 1.5T or 3T scanners (McRobbie *et al.*, 2017). The increased resolution in *ex vivo* brain MRI, ranging from 100 to 500 μm, is attributed to the absence of time constraints during scanning (Dyrby *et al.*, 2013). Notably, resolutions as fine as 50 μm or less can be attained in *ex vivo* tissue specimens (non-hemispheric or whole brain) (Lerch *et al.*, 2017), necessitating longer scan durations, higher magnetic field strengths (e.g. 7T or higher), and specialized gradient systems. The enhanced resolution offered by *ex vivo* MRI compared to *in vivo* human brain MRI is instrumental in investigating the microstructural changes of the brain, particularly in elucidating the impact of psychiatric disorders on structural alterations in specific brain regions. *Ex vivo* MRI of brain tissue can be combined with histopathological analysis to gain a deeper understanding of the pathophysiological mechanisms of neurodegenerative diseases. This approach facilitates a more precise correlation between imaging characteristics and actual tissue alterations, enabling a direct comparison and alignment of MRI parameters with histopathological matrices (Zhou *et al.*, 2020; Yao *et al.*, 2023).

It is worth noting that neuroimaging data itself cannot reveal the molecular changes in human brain pathology and their potential molecular interaction mechanisms. Many aspects of brain structure and function, including brain connectivity, exhibit heritable traits throughout brain maturation (Elliott *et al.*, 2018; Arnatkeviciute *et al.*, 2021). Therefore, characterizing the genetic drivers associated with psychiatric diseases could potentially provide new insights into the complex molecular mechanisms of brain organization. Over the past decade, major advances in high-throughput tissue processing and analysis have made it possible to generate whole-brain transcriptome maps based on brain anatomy (Hawrylycz *et al.*, 2012; Miller *et al.*, 2014). These maps include nearly genome-wide quantitative measurements of transcriptional activity in thousands of tissue samples throughout the brain (Hawrylycz *et al.*, 2012), which has opened up the possibility

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of identifying spatial patterns of gene expression associated with neuroimaging phenotypes (Yao *et al.*, 2024).

Multimodal analysis methods that combine neuroimaging data, histopathological data, and transcriptomics data are promising and developing rapidly, providing researchers with a more complete reference for their work. Yet, the successful integration of these diverse data sets necessitates careful consideration of various data processing and analytical decisions that can influence the ultimate outcomes. Thus, the establishment of optimal procedural workflows is imperative for the field to ensure efficient and replicable progress.

In this review, we introduce a standardized method for *ex vivo* brain multimodal research based on the processing procedures of human brain tissue sampling, processing, storage, and pathological diagnosis in brain banks (Alkemade *et al.*, 2015; Jonkman *et al.*, 2019; Qiu *et al.*, 2019), *ex vivo* MRI techniques for higher image quality (Roebroeck *et al.*, 2019), and the best practice workflow constructed for imaging transcriptomics (Arnatkeviciute *et al.*, 2023). This provides a methodological basis for the application of this process in psychiatric disorders. Furthermore, we explore the applications of *ex vivo* MRI in schizophrenia, major depressive disorder (MDD), and bipolar disorder, highlighting its role in understanding neuroanatomical alterations, disease progression, and the validation of *in vivo* neuroimaging biomarkers.

Technical considerations in *ex vivo* brain MRI

Standard protocol for the collection and fixation of postmortem brain specimen

The procedures for obtaining and preserving brain tissue followed the provisions of the standardized operational protocol for human brain banking in China (Qiu *et al.*, 2019), for which the National Human Brain Bank for Health and Disease of China has developed a pipeline as standard practice (see Fig. 1 for an overview). After removing the brain, the brain is weighed and photographed. The dominant (left) hemisphere is usually dissected according to anatomical structures and stored at −80°C, while the contralateral (right) hemisphere is fixed immediately after being placed into phosphate buffer solution (pH 7.2–7.4) consisting of 6% neutral buffered formalin (NBF). After 3 days the fixed hemisphere will be transferred to 4% NBF, where it will be fixed for more than 4 weeks.

A critical methodological difference emerges in specimen preservation protocols between human and animal *ex vivo* MRI research. Animal neuroimaging studies typically employ premortem transcardiac perfusion fixation (PMI < 60 min). In contrast, in human *ex vivo* studies, the PMI is usually longer, and human brain tissue is usually fixed only by an immersion method (Dawe *et al.*, 2009), with an especially long time to ensure the fixative reaches the depths of the tissues when the volume of the tissue sample is large (Shepherd *et al.*, 2009a). In a study on the effect of long and variable PMI of *ex vivo* human brain tissue (Miller *et al.*, 2011), it was confirmed that PMI showed a significant effect or played a key role in reducing the rate of brain tissue diffusion; that is, for every hour of PMI increase, the diffusion coefficient decreased by (0.1–0.2) × 10^{-4} mm^2/s. The dependence of diffusion parameters on PMI suggests that degradation (such as degradation caused by autolysis before fixation) is the main cause of the differences in diffusion coefficients and relaxation times (T1, T2 value) between *ex vivo* and *in vivo* tissues. For the above reasons, we recommend selection of postmortem human brain samples for MRI analyses with lower PMI whenever possible. Besides, perfusion fixation via the femoral artery about 24 h postmortem, succeeded by immersion postfixation for a period of 30 days, represents an alternative approach to preserve tissue integrity and prevent sample distortion. (Alkemade *et al.*, 2020).

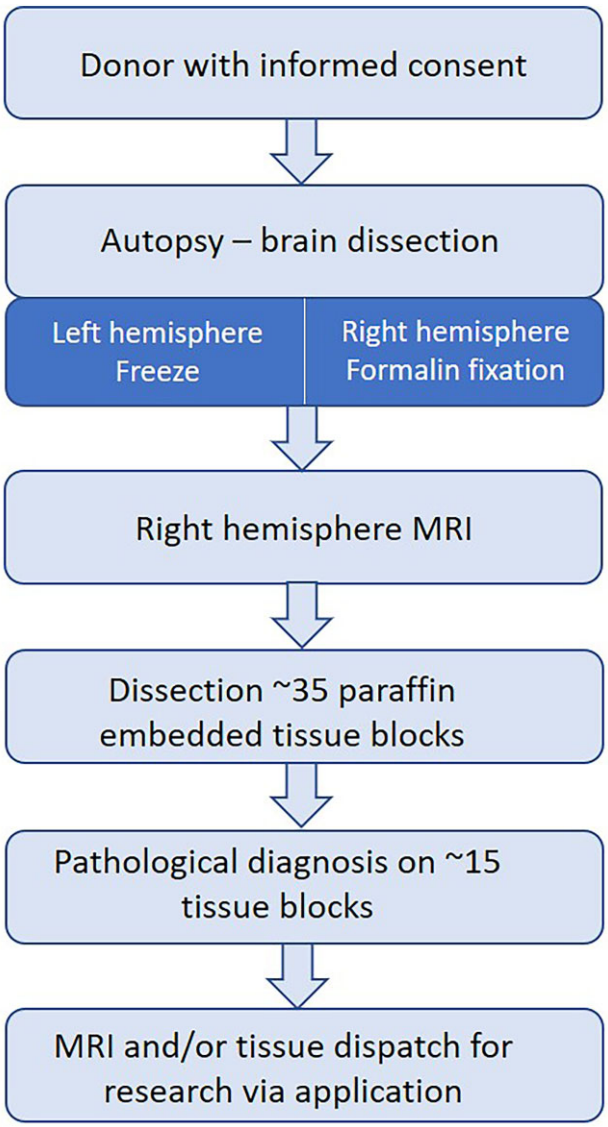

Figure 1: Overview of the pipeline for obtaining and preserving brain tissue.

The biggest obstacle to obtaining high-quality *ex vivo* human brain MRI data is the reduction in T2 and diffusion coefficient of fixed tissue, which can seriously reduce the signal-to-noise ratio (SNR) and contrast of tissue in MRI. This is because low T2 causes rapid decay of spin echo (SE) signals, while a low diffusion rate requires stronger diffusion weighting (longer diffusion time and higher b value), which increases the echo time (TE) of the acquired signal and causes more signal decay. In animal experiments, T1, T2 and diffusion coefficient in *ex vivo* brain tissues are significantly reduced by immersion fixation. For example, Shepherd *et al.* (2009b) showed that compared with fresh unfixed tissue, rat cortical slices fixed by immersion in 4% paraformaldehyde had a 21% decrease in T1 and an 81% decrease in T2. The study also found that long-term washing with saline or phosphate-buffered saline (PBS) to wash away the fixative and rehydrate the sample

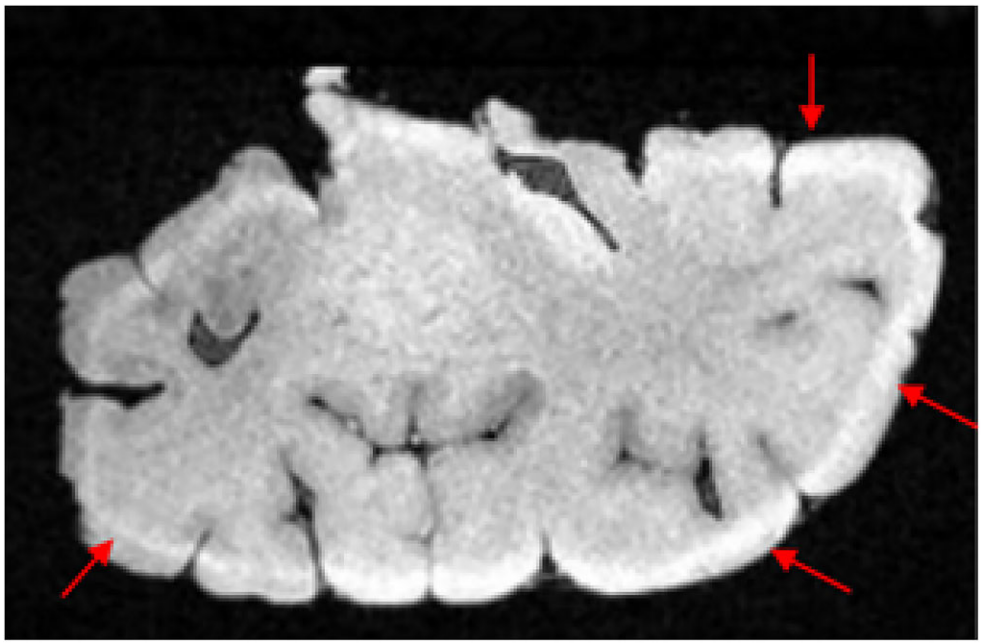

Figure 2: Insufficient PBS soaking time for brain tissue leads to bright bands in T1w images (red arrows).

can completely restore the reduction in T2 value of the fresh unfixed *ex vivo* tissue.

As mentioned above, an effective and common method to improve the diffusion signal is to wash the brain tissue in saline or PBS for a long time to wash off the fixative and rehydrate the sample, which can partially restore the reduction in diffusion coefficient and T2. However, similar to immersion fixation, washing large tissue samples (such as whole brain) with PBS still has the problem of limited passive penetration of the buffer solution into the depths of the tissue (D'Arceuil & De Crespigny, 2007; Miller *et al.*, 2011). As shown in Fig. 2, after soaking the *ex vivo* hemi-brain tissue in PBS solution for 1 week, a clear bright band can be seen on the T1-weighted (T1w) image of the tissue scanned (indicated by the red arrow in the figure). Therefore, we suggest immersing the hemisphere brain sample in water and rinsing with running water for 24 h to restore T1, T2 and the diffusion coefficients. This will result in lower T1, T2 and diffusion coefficients of the tissue during scanning compared to *in vivo* tissue. To obtain good image quality, the scanning parameters need to be adjusted according to the actual situation. For specific adjustments, please refer to Section 'MRI techniques' below.

Following a 24-h rinse with running water, the *ex vivo* human brain hemisphere sample should be gently wiped using gauze. Subsequently, a small quantity of Fomblin (YL VAC25/6, Solvay) is poured into the bottom of a container. The sample is then positioned in the container with the corpus callosum oriented upwards and the temporal lobe downwards. Delicately pressing the sulci and gyri aids in expelling air between the lower surface of the brain hemisphere and the container base. A plastic support may be inserted into a suitable gap to prevent specimen displacement. It is advised to gradually fill the container's inner wall with Fomblin until the brain hemisphere is thoroughly saturated. Common practice is to enhance *ex vivo* MRI scan quality via submerging the specimen in a proton-free liquid with a matching tissue magnetic susceptibility, such as Fomblin or Fluorinert. This technique helps to prevent signal interference from water-based embedding solutions, mitigating clipping or ringing artefacts during low b value (or b0) scanning, and minimizing non-resonant distortions at tissue borders due to matched magnetic susceptibility.

Subsequently, the container is placed in the vacuum machine and vacuumed for 24 h to avoid the bubbles in the brain tissue from affecting the quality of imaging. After the aforementioned procedures, the prepared sample can be transferred to MRI scanning.

MRI techniques

After the *ex vivo* brain sample returns to room temperature (22 ± 0.5°C), it can be scanned by MRI. Imaging is conducted using a ultra-high MRI scanner (e.g. 7T). To ensure the stability during prolonged MRI procedures, the experimental protocol necessitates supplementary immobilization measures beyond the specimen container. Specifically, while the sample maintains static positioning, compressive sponge padding should be implemented peripherally to the containment apparatus. Additionally, weighted sandbags require strategic positioning on the scanning bed to enhance inertial resistance, thereby mitigating potential vibrational artefacts induced by insufficient mass loading of the brain sample. The ultra-high magnetic field enables higher signal strength generation under equivalent imaging parameters. This enhancement directly enhances the SNR, facilitating the acquisition of sufficient signal at a reduced voxel size, thereby enhancing image spatial resolution. Higher magnetic fields lead to more pronounced chemical shift effects, subsequently enhancing resolution between diverse substances in certain scenarios, such as the discrimination between fat and water. Moreover, within the ultra-high magnetic field environment, the material's magnetic susceptibility effect is notably intensified, highlighting differences in magnetic susceptibility and enabling clearer visualization of minute venous structures, bleeding, and calcifications. However, radiofrequency (RF) field inhomogeneity becomes more apparent with increasing magnetic field strength, potentially leading to signal inhomogeneity within the image. Consequently, it is imperative to establish scanning parameters prior to commencing each

new sequence scan, followed by the performance of shimming procedures.

In a 7T environment, for instance, the specific absorption rate (SAR) of RF energy is higher, so the SAR needs to be strictly monitored to avoid tissue overheating. Although *ex vivo* tissue is not affected by SAR limitations, the use of RF energy still needs to be controlled to avoid local overheating of the sample, and ensuring sample temperature stability is necessary during long-term scanning. Temperature fluctuations can affect the magnetic resonance properties of tissue, causing inconsistencies in scan results.

Death and fixation will cause the T1, T2 and diffusion coefficient of *ex vivo* tissue to decrease, thus affecting the image quality. Therefore, the selection of parameters for *ex vivo* human brain tissue magnetic resonance scanning is particularly important. Since the *ex vivo* brain is usually not limited by the scanning time, the SNR can be improved by increasing the number of scan averages, and the TR can be extended to make full use of the T1 and T2 relaxation times. For diffusion-weighted imaging, the b value needs to be increased to increase the sensitivity to diffusion. In addition, if conditions permit, a higher sensitivity coil or multi-channel coil can be used to enhance signal reception and improve image quality. Table S1 provides a comparison of the MRI sequence and parameters used *in vivo* versus *ex vivo*. These adjustments can help improve the MRI scanning quality of *ex vivo* brain tissue, providing clearer images and more accurate structural information. Detailed parameters are provided in Table 1 and the following introduction to the scanning protocol.

Actual flip-angle imaging

Actual flip-angle imaging (AFI) is a technique utilized to precisely assess the flip angle of the RF pulse in MRI. The flip angle is a critical parameter in MRI as it dictates the degree to which the magnetization vector undergoes rotation in response to the RF pulse. This rotation directly influences the ultimate image contrast and signal intensity. Notably, with the advancement to 7T magnetic field strength in scanning, the inhomogeneity of the B1 field becomes more pronounced, leading to potential deviations of the actual flip angle from the designated value. Such deviations can detrimentally impact image quality and subsequent quantitative analysis. Through precise measurement of the actual flip angle, the AFI sequence facilitates the acquisition of data essential for subsequent image reconstruction and correction processes, thereby contributing to the enhancement of overall image quality. Figure 3 shows the B1 field acquired by the AFI sequence. The yellow arrow shows that the B1 field is lower in the edge area.

Structural imaging

T1w images were acquired using a 3D Magnetization-Prepared 2 Rapid Acquisition Gradient Echo (MP2RAGE) sequence, recognized for its ability to produce high-contrast, low-noise T1w images (Marques *et al.*, 2010). The MP2RAGE technique enhances image quality by integrating two gradient echo acquisitions with varying delay times between magnetization preparation steps. By mitigating artefacts stemming from B1 inhomogeneity, MP2RAGE ensures consistent T1w image quality. Notably, this sequence outperforms conventional T1w imaging methods by markedly reducing noise levels, resulting in more spatially uniform images of superior quality. Moreover, MP2RAGE facilitates direct generation of T1 images for streamlined quantitative analysis, unaffected by the T2∗ effect.

T2-weighted (T2w) images were obtained utilizing a 2D fast spin echo (Turbo Spin Echo, TSE) sequence. The TSE technique incorporates multiple 180° inversion pulses to capture numerous

Table 1: Scan parameters of *ex vivo* human brain hemisphere on 7T MRI.

Sequence	AFI	T1w	T2w	T1 mapping	T2 mapping	SWI	Hippo	DW-SSFP
TR[1] (ms)	20/30	6000	6000	4500	2050	46	40	29
TE (ms)	3.45	2.62	31	11	11/23/34/46/57/69	5.62/16.48/27.34/8.2	17	21
TI (ms)		353/1400		60/120/240/480/960				
Flip angle (°)	90	4/5	130	90	90	18	17	29/45/84
Resolution (mm)	2.0 × 2.0 × 2.0	0.5 × 0.5 × 0.5	0.3 × 0.3 × 1.0	1.0 × 1.0 × 2.0	1.0 × 1.0 × 2.0	0.4 × 0.4 × 0.4	0.2 × 0.2 × 0.8	1.0 × 1.0 × 1.0
BW (Hz/Px)	180	240	286	253	163	120	100	402
AT (h:m:s)	1:18	1:11:14	52:09:0	7:54*5	3:43*6	1:20:58	1:44:38	10:25:18

[1]TR: repetition time, TE: echo time, TI: Inversion time, BW: bandwidth, AT: Acquisition time.

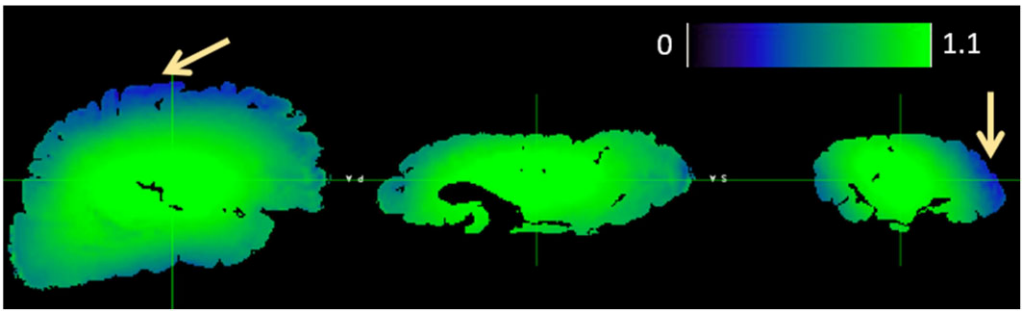

Figure 3: B1 field acquired by the AFI sequence. The yellow arrow shows that the B1 field is lower in the edge area.

echoes within a single TR interval, leading to a notable enhancement in imaging efficiency. By employing multiple 180° pulses, the TSE method proficiently mitigates signal attenuation resulting from the T2∗ effect and preserves a greater extent of T2 contrast data. Given the absence of motion artefacts in *ex vivo* brain tissue and the non-critical constraint on scan duration, the TSE sequence excels in delivering high-contrast, high-resolution images, particularly in the investigation of brain structural features.

T1 and T2 mapping

T1 and T2 quantitative mapping in *ex vivo* MRI analysis is critical for understanding tissue properties, microstructural changes, and pathological processes. The inversion recovery (IR) method was selected for T1 measurement, involving the initial application of a 180° inversion pulse followed by imaging at varying inversion times (TI) to measure the signal recovery to its equilibrium state. Through acquisition of multiple images corresponding to different TI values, the T1 value is determined by analyzing the relationship between signal intensities and TI. Typically, Equation (1) is employed for this analysis:

$$M_z\,(TI) = M_0\left(1 - 2\cdot e^{-TI/T1}\right) \tag{1}$$

where M_z represents the longitudinal magnetization at time TI during inversion recovery, and M_0 signifies the longitudinal magnetization at equilibrium. Subsequent to fitting the data, the T1 value obtained can be utilized to produce a T1 map displaying the T1 relaxation time at each pixel in a grayscale format. This comprehensive approach enables T1 quantitative imaging in MRI, with adjustments of imaging parameters required for different MRI systems and field strengths to ensure the acquisition of high-quality T1 quantitative images.

We have chosen to utilize SE sequence for data acquisition at different TE to facilitate T2 mapping. As TE increases, there is a progressive decrease in signal strength, which correlates with T2 values. The quantitative evaluation of T2 involves the application of curve fitting to the signal decay, commonly employing Equation (2):

$$M_{xy}\,(TE) = M_0\cdot e^{-TE/T2} \tag{2}$$

where M_{xy} represents the transverse magnetization at TE, while M_0 denotes the initial transverse magnetization. Each pixel's signal intensity values at different TEs are utilized to fit the exponential decay formula above to determine the T2 value. Subsequent to the fitting process, the T2 value corresponding to each pixel is utilized to produce a T2 quantitative image, with each pixel's value in the image denoting the T2 at that specific position. Through these procedures, MRI can accomplish T2 quantitative imaging, delivering crucial tissue-specific data for quantitative assessment and diagnosis.

Susceptibility weighted imaging

Susceptibility weighted imaging (SWI) data were acquired using a Multi-Echo Fast Low Angle Shot (ME-FLASH) sequence (Fig. 4a), a technique known for its rapid imaging capabilities facilitated by employing low flip angles and short TR. SWI is sensitive to the presence of substances that cause localized magnetic field inhomogeneities. The high magnetic field strength of 7T can significantly enhance the effect of SWI. Under high magnetic fields, the magnetic susceptibility effect of substances in tissues will be significantly enhanced, making the difference in susceptibility more obvious. For example, iron-containing hemoglobin, deoxygenated hemoglobin, calcium-containing substances, etc., are more sensitive to magnetic fields, thus producing stronger local magnetic field distortion in 7T MRI. This distortion manifests itself as more obvious signal contrast in the image, making structures such as small blood vessels, microbleeds, iron deposits, and calcifications clearer.

These resultant images can be further processed to derive the T2∗ quantitative map and subsequently employed in the computation of quantitative susceptibility mapping (QSM) (Wang & Liu, 2015), to quantify the content of magnetic substances such as iron, calcium, and deoxyhemoglobin in tissues (Bilgic *et al.*, 2012; Reichenbach *et al.*, 2015).

Diffusion weighted imaging

Due to a significant decrease in the diffusion coefficient of *ex vivo* tissues, a higher diffusion weighting is usually required to detect the diffusion of water molecules. Conventional pulsed gradient spin echo (PGSE) diffusion sequences show poor imaging effects in *ex vivo* human brain tissues (Miller *et al.*, 2011). The diffusion-weighted steady-state free precession (DW-SSFP) sequence combines diffusion-weighted imaging (DWI) with steady-state free precession (SSFP), offering advantages such as short TR (suitable for short T2 tissues), high diffusion weighting, and high SNR (Foxley *et al.*, 2014). Nonetheless, the DW-SSFP sequence is greatly affected by motion, rendering it suitable for *ex vivo* brain MRI (Fig. 4b).

Moreover, the DW-SSFP sequence encounters challenges due to its strong reliance on steady-state conditions and susceptibility to signal fluctuations from B0 and B1 inhomogeneities. Consequently, DWI images obtained using DW-SSFP may exhibit varying diffusion weighting throughout the brain, potentially impeding comprehensive brain image analyses. To address this issue, a collaboration with the University of Oxford was ini-

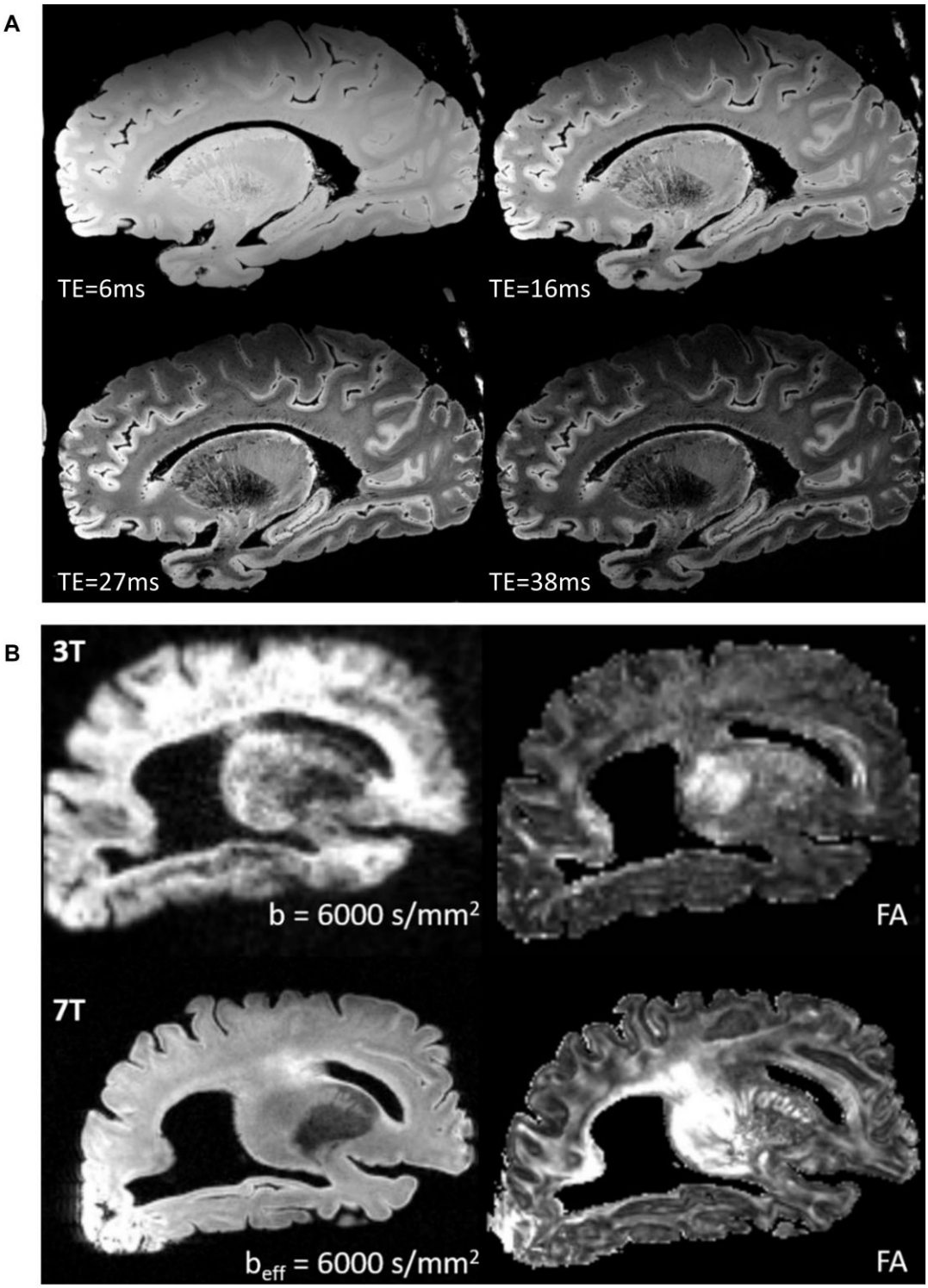

Figure 4: (**A**) ME-FLASH images of *ex vivo* hemisphere. (**B**) Diffusion-weighted images and anisotropy fraction (FA) of *ex vivo* hemisphere.

tiated to formulate a DW-SSFP data acquisition protocol and model grounded on multiple flip angles and effective b values (Tendler *et al.*, 2020). In comparison with images captured with conventional DW-PGSE sequences, this approach upholds superior SNR even at heightened resolutions and effectively mitigates issues arising from RF field inhomogeneities. As illustrated in Fig. 4b, DW-SSFP data demonstrate a greater SNR under identical effective b values, allowing for enhanced resolution imaging.

Neuropathological diagnosis

Neuropathological data are the basis for the study of post-mortem human brain tissues. Accurate pathological diagnosis is demanded for scientific research of neuroscientists on brain bank samples. After the formalin-fixed right hemisphere undergoes scanning with a 7T MRI, it is subjected to a rinsing process to eliminate any residual Fomblin. Subsequently, the meninges are carefully removed, and the hemisphere is assessed and photographed from all directions with a ruler. A coronal section

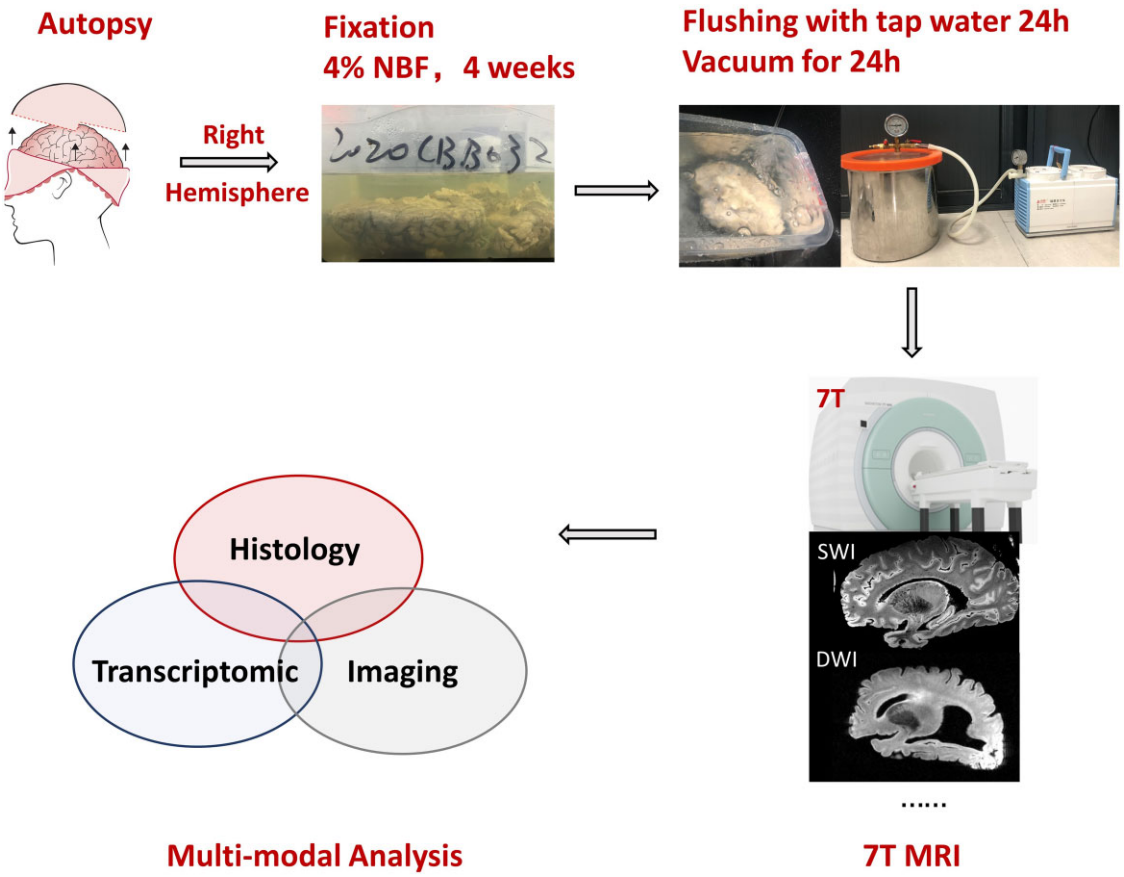

Figure 5: Pipeline of *ex vivo* brain MRI scan and analysis. NBF: neutral buffered formalin; SWI: susceptibility weighted imaging; DWI: diffusion weighted imaging.

is taken caudal of the anterior commissure, followed by the sectioning of 0.5-cm-thick slices in both orientations. The formalin-fixed tissue dissection protocol includes ~35 regions (Jonkman *et al.*, 2019). The formalin-fixed dissected tissue blocks are encased in cassettes and are paraffin embedded.

From the formalin-fixed dissection, 15 regions are selected according to strict standardized protocols in line with BrainNet Europe (BNE), and series of 6-μm-thick sections are cut and mounted onto glass slides. Routine hematoxylin/eosin (HE), silver, and immunohistochemical staining are carried out for the pathological diagnoses. For detailed staining and pathological diagnosis steps, please refer to the standardized operational protocol for human brain banking in China (Qiu *et al.*, 2019), which complies with the latest neuropathological diagnosis standards (Dickson *et al.*, 2009; Deramecourt *et al.*, 2012; Hyman *et al.*, 2012).

Histopathological staining methods, such as HE staining, are commonly utilized for visualizing the microscopic brain tissue structure and alterations in cell morphology, enabling the assessment of neuronal damage, atrophy, and degenerative processes. Immunohistochemistry, on the other hand, employs specific antibodies to target particular protein expressions. In the context of mental disorders, immunohistochemistry serves as a valuable tool for identifying biomarkers associated with neurotransmitters and neuroinflammation (Mabry *et al.*, 2020), shedding light on the underlying biological mechanisms of the conditions. Silver staining, a technique for detecting nerve fiber degeneration, synaptic pathology, and neuronal degeneration, is instrumental in pinpointing neurodegenerative changes linked to mental illnesses by revealing neuronal damage and pathological transformations within the brain tissue.

Figure 5 illustrates the pipeline of postmortem brain specimen collection, fixation, scanning, and data analysis.

Prospects of application in psychiatric disorders

Contemporary psychiatric diagnostics predominantly rely on standardized psychometric assessments, whereas *ex vivo* MRI emerges as a pivotal validation tool for characterizing neuroanatomical abnormalities in mental disorders. Figure 6 summarizes the pathological changes in psychiatric disorders and MRI techniques that can be used to detect the corresponding changes.

Schizophrenia

Schizophrenia represents a debilitating neuropsychiatric syndrome characterized by a tripartite symptom constellation comprising psychotic manifestations (hallucinations, paranoid delusions), deficit syndrome components (anhedonia, asociality, alogia), and neurocognitive impairment domains (working memory capacity reduction). Emerging evidence suggests that multifaceted neurobiological mechanisms underlie schizophrenia.

Contemporary neurochemical models posit that dopaminergic dyshomeostasis within cortico-striatal circuits constitutes a fundamental pathomechanism in schizophrenia disorders

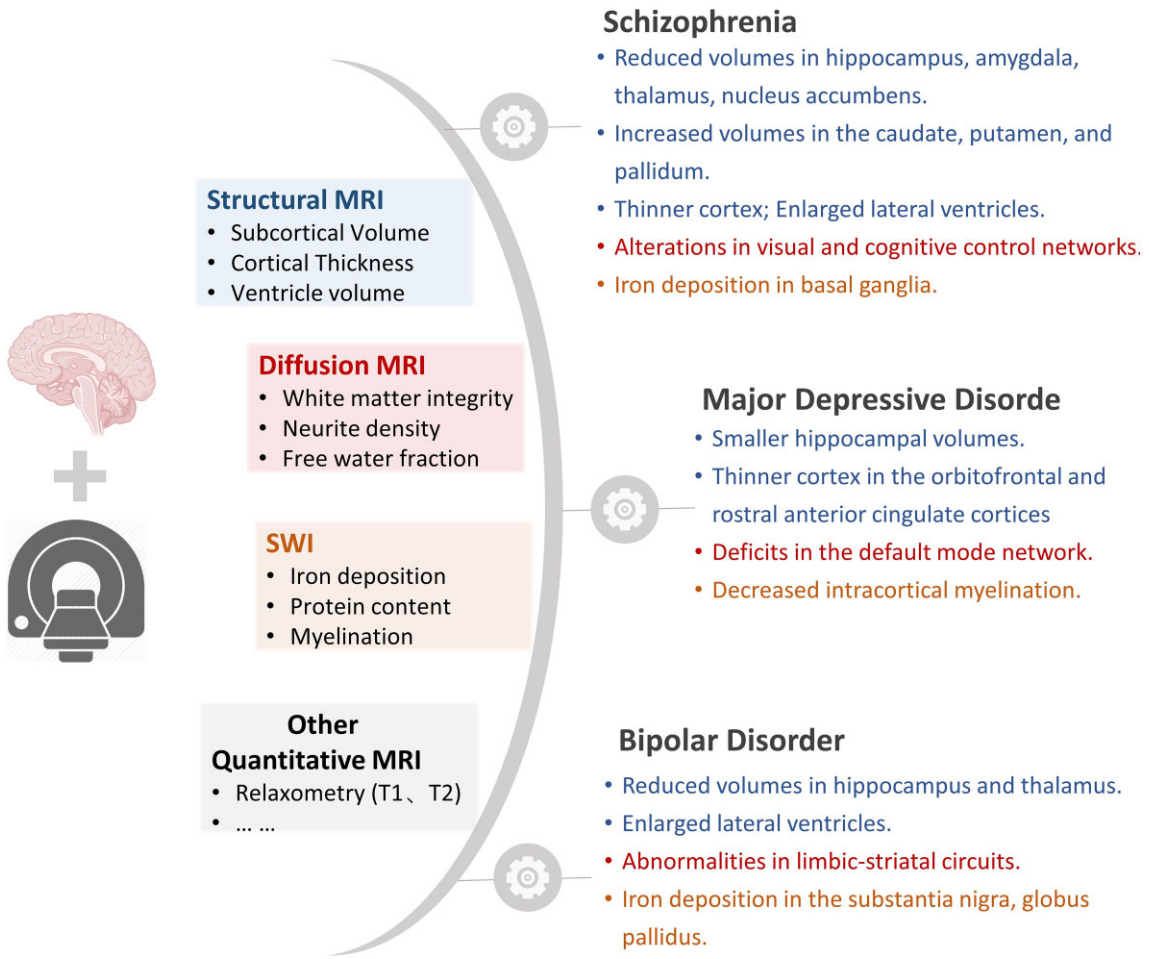

Figure 6: Summary of pathological changes in psychiatric disorders and related MRI techniques. SWI: susceptibility weighted imaging.

(McCutcheon *et al.*, 2019). Recent studies, including molecular imaging and postmortem analyses (van Hooijdonk *et al.*, 2023), have revealed hyperactive dopaminergic neurotransmission in the substantia nigra (SN) of schizophrenia patients. This provides a foundation for understanding how nigral dysfunction contributes to striatal hyperdopaminergia in the disease. By assessing dopaminergic function in the SN, neuromelanin-sensitive MRI has emerged as a potentially valuable clinical biomarker, with recent clinical validations indicating its predictive utility in identifying treatment-refractory schizophrenia subtypes (van der Pluijm *et al.*, 2024).

Complementary insights from proton magnetic resonance spectroscopy (^{1}H-MRS) studies provide indirect evidence of neurotransmitter system interactions that may potentiate dopaminergic imbalance (Kumar *et al.*, 2020). Although ^{1}H-MRS itself does not directly measure dopamine, its capacity to quantify neurometabolic concentrations enables detections of glutamate and other neurotransmitter systems, revealing metabolic changes associated with neurotransmitter imbalances.

The emerging paradigm of histology combines ultra-high field MRI datasets with multi-omics profiling to bridge molecular and systems-level analyses. This integrative approach has identified novel biomarkers and potential therapeutic targets within the SN (van Wijk *et al.*, 2020), offering novel insights into the neurobiological roots of schizophrenia. Nonetheless, such investigations remain scarce, with the current literature limited to a combination of immunohistochemistry and positron emission tomography (PET) analysis on postmortem cohorts (Howes *et al.*, 2013).

Schizophrenia is also characterized by a range of structural abnormalities which can be detected by MRI. Meta-analytical evidence (Howes *et al.*, 2023) indicates that schizophrenia is characterized by decreased volumes and thickness, accelerated atrophy over time, and abnormal gyrification patterns in cortical regions. These alterations are particularly evident in regions such as the frontal cortex, anterior cingulate cortex, temporal cortices, and hippocampus. Postmortem MRI analysis of 96 decedents revealed a significant 9.5% reduction in hippocampal volume among schizophrenia patients compared to controls (Busch *et al.*, 2019). Other postmortem investigations further demonstrated decreased synaptic density in the anterior cingulate cortex, frontal cortex, and hippocampus of schizophrenia cohorts (Osimo *et al.*, 2019).

Furthermore, MRI findings can be correlated with histopathological data (Alkemade *et al.*, 2015, 2023), such as neuron density and gliosis, to achieve a more thorough understanding of the pathological root causes of the disease. Advances in high-resolution imaging techniques have enabled more precise visualization of these brain abnormalities, providing a clearer picture of how schizophrenia affects brain structure. By bridging the gap between neuroimaging and histological analysis, *ex vivo* MRI plays a pivotal role in the discovery of biomarkers and potential therapeutic targets for schizophrenia.

Major depressive disorder

As one of the most prevalent mental illnesses, MDD has been increasingly associated with altered cortical gray matter morphology (Schmaal *et al.*, 2017), despite an incomplete understanding of its neuropathological basis. Neuroimaging studies have identified the prefrontal and anterior cingulate cortices as key areas showing such morphological alterations, aligning with the critical roles of these regions in regulating salience processing, emotional responses, and decision-making processes (Monosov *et al.*, 2020; Pizzagalli & Roberts, 2022). Meta-analytical investigations conducted by the ENIGMA MDD Consortium have specifically emphasized the cortical thinning observed in the orbitofrontal and rostral anterior cingulate cortices as robust indicators of MDD (Schmaal *et al.*, 2017). Nevertheless, while *in vivo* morphometric measurements can detect alterations in the cortical boundaries, the precise microstructural correlates underlying these changes remain elusive. Quantitative relaxometry mapping at ultra-high field has the capacity to partially characterize the microstructural properties of intracortical myelin. This advanced technique has further revealed associations between such myelin-related microstructure and the MDD condition (Heij *et al.*, 2024). Importantly, both MDD diagnosis and symptom severity were found to be associated with decreased myelination in the lateral orbitofrontal cortex. This region plays a crucial role in processing negative affect and the emotional experiences of sadness. These findings tentatively suggest that demyelination may contribute to the large-scale cortical disintegration and disrupted neural circuits in MDD.

Iron accumulation in the basal ganglia region has been proposed as a contributor to psychiatric disorders (Yao *et al.*, 2017). In a recent *in vivo* study, a significant relationship between subcortical susceptibility and volume was observed in the nucleus accumbens of the MDD group. This observation indicated abnormalities in myelination and the dopaminergic system related to iron deposition (Shibukawa *et al.*, 2024). Nonetheless, there is a lack of research that specifically employs quantitative susceptibility mapping to analyze the postmortem brains of individuals diagnosed with MDD. *Ex vivo* MRI offers a valuable avenue to explore the distribution and significance of iron in the brains of MDD patients, and to carry out histological validation, thus establishing a potential connection between irregular iron levels and manifestations of depression.

Bipolar disorder

Bipolar disorder is a chronic mental health condition with severe and recurrent mood fluctuations, manifesting as distinct phases of mania or hypomania, as well as depression. Although the pathogenesis of bipolar disorder has not been fully elucidated, neuroinflammation has been investigated as a potential cause (Naaldijk *et al.*, 2016). A postmortem study has highlighted decreased neuronal and glial densities and smaller neuron size in frontal and subcortical areas of bipolar disorder patients (Gigante *et al.*, 2011), and hypothesized that these cellular abnormalities might be associated with enhanced apoptosis, instigated by oxidative stress and the overproduction of reactive oxygen species, potentially representing a pivotal pathogenic mechanism. The associations between immune characteristics and functional or structural MRI modifications indicate brain regions engaged in affective and somatomotor processing. Neuroimaging–immunological convergence analyses have demonstrated a tendency towards a negative correlation between peripheral inflammatory markers and the volume of certain brain regions (Saccaro *et al.*, 2023). Moreover, the evidence of neuroinflammation in postmortem brain samples of bipolar disorder has been reviewed and evaluated (Giridharan *et al.*, 2020), focusing on markers of microglia, astrocytes, cytokines, and other inflammatory indicators. While some studies suggest evidence of inflammation in bipolar disorder postmortem brain samples, the variability in findings, influenced by factors such as PMI, brain region, and treatment history, prevents a definitive conclusion, highlighting the need for more consistent research to clarify the role of inflammation in bipolar disorder pathology (Sneeboer *et al.*, 2019).

Although MRI lacks cellular-resolution specificity, it enables *in vivo* quantification of neuroinflammatory biomarkers. Diffusion kurtosis imaging has been used to study neuroinflammation in schizophrenia (McKenna *et al.*, 2019), reporting increased mean kurtosis in patient groups relative to healthy control subjects. Increased kurtosis may be indicative of heightened inflammatory processes. Free-water (FW) imaging has been used to study neuroinflammation across several disorders (Zhang *et al.*, 2023). Recent research indicated that elevated peripheral proinflammatory signaling of interleukin-6 and tumor necrosis factor α were correlated with widespread increased brain FW in patients with schizophrenia (Di Biase *et al.*, 2021). These results support prior findings of cytokine dysregulation and FW abnormalities in schizophrenia, establishing a putative mechanistic link between systemic inflammation and perivascular space dilation. Considering the growing evidence for a role of inflammation in depression, additional FW studies in larger depression cohorts, incorporating supplementary inflammation markers from alternative methodologies, are necessary before definitive conclusions regarding the utility of FW imaging in detecting neuroinflammatory processes in depressive disorders can be established. Postmortem immunohistochemistry enables the quantification of diverse immune cell populations and related processes. When conducted within a region demonstrating increased levels of the MRI marker, correlations between the MRI marker and specific immune processes can be confirmed. However, such investigations are currently lacking (Oestreich & Sullivan, 2022).

Integration of *ex vivo* MRI with other techniques

Histopathology

Ex vivo MRI offers an invaluable tool for bridging the gap between imaging and histopathology. By combining high-resolution MRI with histological techniques, researchers can correlate structural changes seen on MRI with underlying cellular and molecular alterations (Alkemade *et al.*, 2023; Yao *et al.*, 2024). This integrated methodology provides a more thorough and exhaustive understanding of disease mechanisms and allows for the validation of MRI biomarkers.

The joint analysis of MRI and histological data (Fig. 7b) enables a comprehensive assessment of the precision of MRI models and the verification of their ability to faithfully represent the anatomical and pathological attributes of *ex vivo* brain tissue through comparison with stained sections. These assessments yield valuable insights for refining model efficacy, thus enhancing the clinical and research utility of MRI imaging. In postmortem human brain research, accurately aligning thick-section MRI datasets (typically acquired at 0.2–1 mm slice thickness) and thin-section histological preparations (commonly sectioned at 6 μm) presents substantial methodological complexities. This difficulty represents a common hurdle encountered in the majority of neuroimaging studies of this nature (Kelm *et al.*, 2016; Zhou *et*

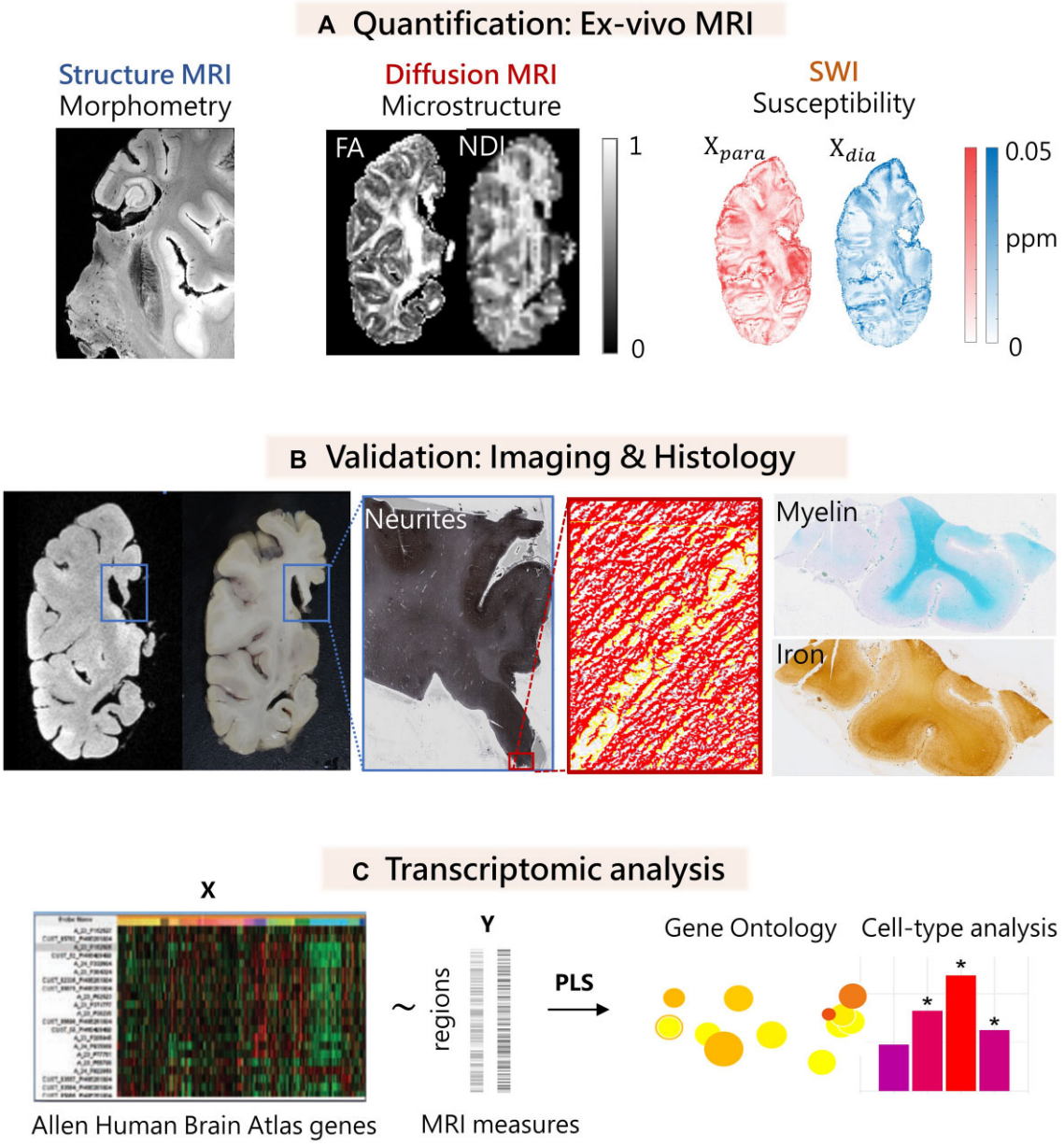

Figure 7: Integration of *ex vivo* MRI with histology and genetics.

al., 2020). Owing to the absence of a reliable method for registering the hemisphere image to a standard atlas, the selection of a region of interest (ROI) on brain specimens has generally been performed manually. This manual approach inherently introduces a degree of subjectivity and potential bias into the process. Notably, a comparison of morphological traits (e.g. sulci, gyri, boundary morphology) in stained sections and MRI images is conducted to assess their alignment (Zhou *et al.*, 2020; Yao *et al.*, 2024). In future studies, it is anticipated that the exploration of 3D histological data will become more prevalent, thereby enhancing the registration of MRI and histological data.

Pathological attributes (e.g. lesion area, cell density) in MRI images and stained sections are compared, with the latter serving as the gold standard for verifying the lesion detection capability of MRI models. Statistical techniques such as Pearson correlation analysis, t-tests, and ANOVA are employed to quantitatively evaluate disparities in pathological characteristics between corresponding regions in stained sections and MRI images.

Genetic and molecular analysis

Psychiatric conditions represent a group of multigenetic disorders with complex etiology that contribute significantly to elevated rates of morbidity and mortality (Ramaker *et al.*, 2017). Despite their distinct clinical presentations, the convergence of symptoms of these disorders strongly implies the presence of shared molecular dysregulations. Therefore, elucidating the genetic determinants of neuroimaging phenotypes associated with psychiatric illnesses may provide valuable insights into the complex molecular processes that govern brain structure and function (Arnatkeviciute *et al.*, 2023).

Ex vivo MRI also enables the integration of genetic and molecular analysis (Fig. 7c), facilitating the identification of gene–environment interactions that contribute to brain abnormalities in psychiatric and neurodegenerative disorders. Cross-modal analysis serves as a powerful tool for deciphering spatial covariance patterns existing between regional neuroimaging metrics (such as cortical thickness, T2 relaxation) and transcriptomic

profiles (Arnatkeviciute *et al.*, 2023). For instance, in our prior study, a partial least squares (PLS) analysis was employed to examine the neuroimaging features of each brain region and the gene expression levels within those regions. This analysis effectively reveals the strength of the relationship between the imaging data and gene expression. Subsequently, genes whose expression patterns exhibit a significant correlation with the neuroimaging data are then chosen for enrichment analysis (Yao *et al.*, 2024).

By correlating imaging data with gene expression patterns regionally, researchers can identify biomarkers associated with disease susceptibility and progression. For example, a study used a multiscale approach to explore the neurobiological basis of MDD by integrating brain imaging, genetic data, and transcriptional information from cortical samples (Anderson *et al.*, 2020). The results showed that depression-related imaging phenotypes are linked to down-regulated gene expression in somatostatin interneurons and astrocytes, with GWAS (genome-wide association study) polygenic risk for depression enriched for genes expressed in interneurons, highlighting key molecular pathways involved in brain structure and function of depressive patients. Another study analyzed multi-modal MRI data from a large sample of 1660 MDD patients and 1341 controls to explore cortical functional and structural abnormalities in MDD (Zhu *et al.*, 2024), linking these changes to gene expression patterns. The results revealed localized impairments in cortical function and global connectivity disruptions, with specific genetic pathways, such as those related to endoplasmic reticulum stress and neurotransmitter function, which are correlated with these cortical alterations, offering insights into the transcriptomic underpinnings of MDD vulnerability.

Discussion

Despite its advantages, *ex vivo* MRI presents several challenges and limitations. The preservation of brain tissue for *ex vivo* MRI scanning is crucial for obtaining high-quality images. Inadequate preservation measures may result in the degradation of artifacts and compromise the structural integrity. It is advisable to utilize samples with a PMI of less than 24 h for analytical purposes. Significant reductions in diffusion coefficients with hourly decreases ranging from 0.01 to 0.02 $\times$ 10^{-3} mm^2/s, and T2 relaxation times may affect image quality (Miller *et al.*, 2011). Besides, prolonged agonal phases (e.g. hypoxia) may induce tissue acidosis and autolysis, altering MRI parameters such as T2 relaxation times. Acute versus chronic causes (e.g. trauma vs. neurodegenerative disease) may differentially affect postmortem tissue stability. We recommend stratifying analyses by cause of death in future studies. Chronic use of psychotropic medications (e.g. antipsychotics, antidepressants) may induce neuroplastic changes or neurochemical alterations (Correll *et al.*, 2015). Thus, the significance of detailed medical reports is recognized, and it is advisable to collect comprehensive information in future research protocols. *Ex vivo* investigations commonly rely on postmortem tissue, a practice that restricts sample size and has the potential to introduce biases related to factors such as age, sex, and other demographic variables. The scarcity of postmortem brain samples from individuals with psychiatric conditions poses a significant limitation on research endeavors in this field.

Ex vivo MRI has emerged as a distinctive and potent instrument in enhancing our understanding of cerebral abnormalities associated with psychiatric disorders. Through the provision of high-resolution images of postmortem brain tissue, *ex vivo* MRI facilitates the exploration of microstructural alterations and neuropathological attributes that frequently elude detection through *in vivo* methods. Despite persisting challenges, the continued development of *ex vivo* MRI techniques, along with their integration with diverse imaging modalities and molecular investigations, holds great promise for improving the diagnosis, treatment, and understanding of psychiatric and neurodegenerative disorders.

Open-science collaboration has emerged as a major global research trend. *Ex vivo* data, particularly in neuroscience, are uniquely valuable within this framework. The National Health and Disease Human Brain Tissue Resource Center (http://zjubrainbank.zju.edu.cn/) sets a precedent with its ethical guidelines that ensure privacy protection and facilitate effective scientific collaboration.

In future studies, a greater emphasis should be placed on enhancing the integration of *ex vivo* MRI with histological and genetic analyses. Our review revealed that many investigations have primarily conducted qualitative comparisons between MRI and histological findings, lacking a systematic correlation between the two types of measurements. Improved correlations between imaging and histology, as well as imaging and pathology, could offer more robust evidence for the potential clinical utility of *ex vivo* MRI. Furthermore, further investigation is warranted into the genetic implications of *ex vivo* MRI results. By conducting multimodal analyses on *ex vivo* human brains, researchers can combine various imaging modalities to gain a more comprehensive understanding of the molecular, cellular, and tissue-level underpinnings of psychiatric disorders. This approach has the potential to facilitate the identification and validation of novel imaging biomarkers, consequently advancing clinical diagnostic and therapeutic practices. The implementation of standardized methodologies can aid in the optimization of technologies and expedite the translation of research findings into clinical applications.

Supplementary data

Supplementary data are available at *PSYRAD Journal* online.

Author contributions

Junye Yao (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing), Zihan Zhou (Data curation, Investigation), Qiqi Tong (Data curation, Investigation), Lingyu Li (Investigation), Jintao Wei (Investigation), Jing Lu (Writing – review & editing), Shaohua Hu (Writing – review & editing), Aimin Bao (Supervision, Writing – review & editing), and Hongjian He (Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing).

Conflict of interests

The authors declare no conflicts of interest.

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